

CLAIMS

1. A method of detecting the presence of contaminating mycoplasma in a test sample comprising:
 - (i) providing a test sample;
 - (ii) detecting and/or measuring the activity (B) of acetate kinase and/or carbamate kinase in the test sample, and said activity being indicative of the presence of contaminating mycoplasma; and
 - (iii) identifying the test sample as contaminated with mycoplasma on the basis of detection and/or measurement of said activity in step (ii).
2. The method of claim 1 further comprising the following steps performed after step (ii) but before step (iii):
 - (iia) obtaining acetate kinase and/or carbamate kinase activity information (A) detected and/or measured in a corresponding control sample; and
 - (iib) comparing the activity detected and/or measured in the test sample (B) with that in the control sample (A); wherein the test sample is identified as contaminated with mycoplasma in step (iii) if the activity (B) detected and/or measured in the test sample in step (ii) is greater than that of the control sample (A) in step (iia), that is, the ratio $\frac{B}{A}$ is greater than one.

3. The method of claim 1 or 2 wherein detecting and/or measuring the activity (B) of acetate kinase and/or carbamate kinase in the test sample in step (ii) and/or obtaining acetate kinase and/or carbamate kinase activity information (A) in a corresponding control sample in step (iia) comprises detecting and/or measuring the appearance and/or disappearance of one or more of the substrates and/or one or more of the products of the following reactions:
 - 10 (Ri) $\text{acetyl phosphate} + \text{ADP} \xrightleftharpoons{\text{acetate kinase}} \text{acetate} + \text{ATP}$
 - (Rii) $\text{carbamoyl phosphate} + \text{ADP} \xrightleftharpoons{\text{carbamate kinase}} \text{ammonia} + \text{carbonate} + \text{ATP}.$
- 15 4. The method of claim 3 further comprising the step of releasing mycoplasma cellular contents into the sample by treatment of the test sample with a mycoplasma lysis agent that is performed after step (i) but before step (ii).
- 20 5. The method of claim 4 wherein the lysis agent is a detergent.
6. The method of claim 5 wherein the detergent lysis treatment is not capable of lysing bacterial cells.
- 25 7. The method of claim 6 wherein the corresponding control sample is the same as the test sample prior to mycoplasma lysis treatment.
8. The method of claim 2 wherein the corresponding control sample is the same as the test sample but the obtention of
 - 30 detection/measurement for the test sample activity information is

carried out after a time interval following the obtention of detection/measurement information for the control sample.

- 5 9. The method of claim 8 wherein the time interval is at least approximately 30 minutes.
10. The method of claim 1 or 2 wherein the detecting and/or measuring step comprises detecting and/or measuring ATP.
- 10 11. The method of claim 10 wherein the ATP is detected and/or measured by a light-emitting reaction.
12. The method of claim 11 where the light emitting reaction is a bioluminescent reaction.
- 15 13. The method of claim 10 wherein ADP is added to the test sample prior to the detecting and/or measuring step (ii).
14. The method of claim 1 or 2 wherein a mycoplasma substrate (MS) reagent is added to the test sample prior to the detecting and/or measuring step (ii), the MS reagent comprising: acetyl phosphate or a precursor thereof and/or carbamoyl phosphate or a precursor thereof.
- 20 14. The method of claim 1 or 2 wherein a mycoplasma substrate (MS) reagent is added to the test sample prior to the detecting and/or measuring step (ii), the MS reagent comprising: acetyl phosphate or a precursor thereof and/or carbamoyl phosphate or a precursor thereof.
- 25 15. The method of claim 14 wherein the precursor of acetyl phosphate is acetyl-CoA.
16. The method of claim 14 wherein the precursor of carbamoyl phosphate is citrulline and/or ammonia.

17. The method of claim 13 wherein the control sample is all or an aliquot of the test sample to which a mycoplasma reagent has not been added.
- 5 18. The method of claim 14 wherein the control sample is all or an aliquot of the test sample to which a mycoplasma reagent has not been added.
- 10 19. The method of claim 2 wherein the control sample has been shown to be free from mycoplasma by a separate method.
20. The method of claim 10 wherein the control sample has been shown to be free from mycoplasma by a separate method.
- 15 21. The method of claim 14 wherein the control sample has been shown to be free from mycoplasma by a separate method.
- 20 22. The method of claim 19 wherein the control sample has been shown to be free from mycoplasma by one or more of PCR testing, DNA fluorescence staining, or mycoplasma culture method.
- 25 23. The method of claim 20 wherein the control sample has been shown to be free from mycoplasma by one or more of PCR testing, DNA fluorescence staining, or mycoplasma culture method.
- 30 24. The method of claim 21 wherein the control sample has been shown to be free from mycoplasma by one or more of PCR testing, DNA fluorescence staining, or mycoplasma culture method.
- 30 25. The method of claim 1 or 2 wherein the test sample and/or control sample is a cell-culture sample.

26. The method of claim 25 wherein cells in the cell-culture sample are mammalian cells, preferably adherent cells, such as Vero, MRC5, HUVEC, BSMC, NHEK, MCF-7, AoSMC, A549, HepG2, FM3A, PC12, ARPE-19, CHO and COS cells, and/or adherent primary cells isolated from animal source.
27. The method of claim 25 wherein the mammalian cells in the cell-culture sample grow in suspension, such as K562, U937, HL-60, Cem-7, and Jurkats plus primary cell types such as leukaemic blast cells.
28. The method of claim 25 where the cell culture is a culture of plant cells.
29. The method of claim 25 where the cell culture sample is a sample which is derived from a cell culture but is itself substantially free of cellular material.
30. The method of claim 1 or 2 wherein the test sample and/or control sample consists of a cell-free reagent.
31. The method of claim 30 where the cell-free reagent is trypsin.
32. A process for treating a cell culture to remove mycoplasma contamination comprising:- treating a mycoplasma contaminated cell culture with an agent to remove and/or destroy mycoplasma; and subsequently testing a sample from the culture for mycoplasma contamination using the method of claim 1 or 2; if necessary, repeating the process of treating one or more times until mycoplasma contamination is not detected in a sample.

33. A method of detecting the presence of mycoplasma in a test sample, comprising the following steps:-
- 5 (i) providing a test sample;
 - (ii) without adding an exogenous reagent (e.g. substrates for kinase activity) to convert ADP to ATP, detecting or measuring ATP in the test sample using a bioluminescent reaction to obtain an ATP and/or light output measurement (A);
 - 10 (iii) obtaining an ATP and/or light output measurement (B) from a corresponding control sample;
 - (iv) comparing the ATP and/or light output measurement ratio $\frac{B}{A}$; and
 - (v) identifying the test sample as contaminated with mycoplasma in the event that the ratio $\frac{B}{A}$ is greater than one.
- 15 34. The method of claim 1, 2 or 33 wherein the method includes a step of passing the test sample through a filter which retains bacterial cells.
- 20 35. A kit for use in the detection of mycoplasma contamination which comprises the following:
- (i) acetyl phosphate or a precursor thereof and/or carbamoyl phosphate or a precursor thereof;
 - 25 (ii) ADP at an amount in excess to drive the enzymatic reactions in the direction of ATP formation;
 - (iii) one or more agents for lysing mycoplasma.
36. The kit of claim 35, wherein the agents for lysing mycoplasma comprises a detergent.
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37. The kit of claim 35 further comprising means for detecting and/or measuring ATP by a light-emitting reaction.
- 5 38. The kit of claim 37 wherein said means comprise a mycoplasma detection reagent (MDR) which includes magnesium acetate, inorganic pyrophosphate, bovine serum albumin, luciferin luciferase, ADP and AMP.
- 10 39. The kit of claim 35, 36, 37 or 38 wherein reagents are provided in a lyophilised condition.
40. The kit of claim 39 which further comprises a mycoplasma assay buffer (MAB) in which lyophilised reagents can be reconstituted.
- 15 41. The kit of claim 40 wherein the buffer maintains a pH of approximately 7.5.
42. The kit of claim 37 further comprising a luminometer, which is preferably a hand-held luminometer.
- 20 43. The kit of claims 35 further comprising a bacterial filter.